IV. *On the Process of Calcification in Enamel and Dentine.*

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Plates 7 and 8.

In considering the process of calcification in dentine and enamel I have endeavoured to carry further my investigations as to the structure and development of dentine commenced in 1892,* the object of that paper having been to show the existence of a connective tissue foundation derived from the tooth pulp, in which calcification takes place much as it does in membrane bone. The actual mode of deposition of the lime salts I did not then consider, except to express the opinion that the calcification took place by a process of secretion from the cells of the pulp. Since that date and following the previous investigations of von Ebner and others, the conversion theory of the formation of dentine has been to a great extent abandoned, this conversion theory being that the odontoblast cell became actually converted into dentine matrix, its centre remaining uncalcified as the soft fibril, and the rest of the cell forming in different degrees of calcification the Neumann's sheath and the matrix. The view held in this paper and by numerous histologists at the present day is that the cells of the pulp secrete a material which calcifies, they themselves not entering into the calcified substance, but receding farther and farther into the pulp as calcification advances, and the fibril becomes more and more elongated.

The odontoblast cell has had many functions assigned to it, one of them being that it is a nerve end-organ, and that its prolongation, the dentinal fibril, conducts sensation from the dentine. As Mr. Chas. Tomes says, writing in 1904 (1), "the difference of opinion as to the function of the odontoblast cells never can be finally settled until the nerve endings of the pulp are finally demonstrated." As I hope it is now satisfactorily proved that true nerve fibres are distributed to the dentine,† the ground is cleared for a further investigation of the real nature and functions of the cells and dentinal fibrils. We can no longer look upon the odontoblasts as being in any sense nerve end-organs, or consider their processes, the dentinal fibrils, as transmitters of sensation from the hard substance of the dentine. From their position and structure we may confidently consider them to be actively engaged in

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the formation of dentine, and I shall endeavour to produce evidence of this and of the way in which their functions are carried out, drawing special attention to certain processes of a purely physical nature which are concerned in calcification, and their co-ordination with the action of the living cells.

In order to study the process of dialysis of lime salts under purely physical conditions, I made some experiments in which particles of sulphate of copper were dropped into a solution of silicate of potash in water containing lime salts and examined under the microscope.

In the tubes of the osmotic growth so produced delicate transverse membranes occasionally appeared. After an interval of several days large spherical colourless bodies formed on the distal side of the membranes; these were exactly similar in appearance to many of the calcospherites produced artificially in albumen. They would appear in this instance to be spheres of silicate of lime formed in a colloidal medium by the dialysis of their constituent salts through the membrane, showing that the passage of salts through these osmotic membranes does take place. These bodies behave to polarised light exactly as do the similar spherites produced in albumen.

The penetration of osmotic membranes by calcium salts has necessarily an important bearing upon the nature of calcification in enamel and dentine, as the process would appear to depend to a great extent upon the dialysis of the lime salts. The matrix of both tissues being a substance to a considerable extent removed from the pale of nutrition, we should expect physical processes to play a large part. While, however, the *rôle* of purely physical processes has to be taken into consideration, it must be remembered that, as Prof. PHILIP says (2), "the behaviour of the living cell membrane towards the substances with which it comes in contact is in many cases incapable of interpretation on a purely physical basis." This is instanced by the altered conditions in dead cells which permit the penetration of stains to which they are impervious when living, and also by the fact, which he instances, that the salts contained in the blood corpuscle differ from those in the plasma, the corpuscles containing an abundance of potassium and phosphate, while the plasma shows a large proportion of sodium and chloride, and little of the potassium and phosphates. These facts show that "a purely physical theory of the exchanges which take place across a living membrane is inadequate; there is a physiological permeability as well as a physical permeability."

The Artificial Production of Calcospherites.

In the year 1858 RAINEY published his work on molecular coalescence (3), showing that lime salts were deposited in colloidal substances, not in a crystalline, but in a globular form, the globules having a very definite structure and arrangement. He also showed that in many animal organisms, as mollusca and crustacea, similar forms appeared during the process of calcification of the shell.

Both RAINEY and HARTING (4) found that when calcium phosphate was present in the solutions in excess of the carbonate, globular bodies were not formed, but the deposit was crystalline; but if there was only a small proportion of phosphate to carbonate, larger and more perfect spheres were produced than with carbonate alone.

This observation has a very important bearing on the subject of the present paper, as in completed dentine and enamel the phosphates are largely in excess.

On repeating the experiment of Prof. HARTING with albumen I found that two main forms of calcospherites may conveniently be distinguished, those in which radial striæ are most evident and those especially marked by concentric lamellæ or rings (Plate 7, fig. 5; Plate 8, fig. 5), although there are many which show both structures.

In many of the larger radial spherites, beside the delicate radial striæ, wider spaces are seen extending from the centre to the circumference, of an irregular shape and evidently indicating a splitting or dividing of the spherite into sections. This is frequently seen in the disintegrating globules in enamel, presently referred to.

In studying the process of calcification in the crustacea, I examined the forming shell of the crab, of the prawn, the crayfish, and the small Norway lobster (*Nephrops Norvegicus*), a species which I do not think has been previously examined for this purpose.

The calcospherites in the carapace of the prawn (Plate 7, fig. 11) are of the radial type and of particularly elegant forms; in the crayfish they are chiefly in the form of compressed spirals or helices, while in *Nephrops* they are radiate and of very definite floral form (Plate 7, fig. 13) and show very clearly the breaking up of these flower-like elements in the production of the shell. In a ground section of the forming shell of the crab (Plate 7, fig. 12) it can be seen by dark-ground illumination that the lines in the detached calcospherites form the transverse striæ visible in the formed shell.

It is noticeable, as I shall describe in dentine and enamel, that there appears to be a special type of calcospherite in each of these different structures.

I think it is evident in all these examples that the shell is ultimately formed by the disintegration of the calcospherites, which may take place either previous to their incorporation with its substance or after that incorporation has taken place.

In all these organisms which I have examined, in crustacea, mollusca, and brachiopoda, there is a distinct finely fibrillar basis substance in which calcification takes place, which appears to serve as a support to the material secreted by the animal. This fibrillar substance is of a clear, apparently structureless, homogeneous nature, and does not appear in any way to determine the actual arrangement of the calcareous particles, but simply seems to serve as a binding material. It is not a true connective tissue and shows no connection with cells.

Calcification in the Dentine.

In young growing teeth, as is well known, there is a portion of the matrix forming a marginal band between the calcified dentine and the odontoblast cells.

This layer, the odontogenetic zone, appears to consist of the collagenous basis substance of the dentinal matrix in which the deposit of lime salts takes place. As shown in a former paper (5), fine connective tissue fibres from the pulp can be seen to become incorporated in this material, although from their transparent nature and their index of refraction so nearly approaching that of the material in this zone, they can seldom be seen in its substance; but, as stated in the paper above referred to, they are occasionally revealed in the formed matrix of dentine by the decalcifying action of acids in caries.

The uncalcified portion of the matrix, unlike the calcified part, takes the stain readily, and the advancing calcification is seen encroaching upon it in the form of rounded masses of the lime salts, some of the calcospherites lying free in the surrounding uncalcified material (Plate 7, fig. 3). The calcospherites in this advancing layer are seen to be quite clear and to exhibit no radial or concentric markings.

When a young tooth is decalcified, the rounded contours of the calcifying border have exactly the same appearance as in the calcified tooth—they appear structureless and consist of the calcoglobulin basis of the spherites, which takes stains readily. The well known interglobular spaces in imperfectly developed dentine show very clearly the advance and coalescence of these globular bodies on the basis substance.

Fig. 4, Plate 7, is from a section at the side of the root of a human premolar in which the end of the root is still open and there is a very active deposition of lime salts in its neighbourhood.

It will be seen that the space between the opposite sides of the pulp cavity is crowded with calcospherites in all stages of coalescence. Comparison with calcification in the crab-shell shows that the same process of coalescence is in progress, but in the tooth the calcospherites do not show the striæ which are so evident in the similar bodies in the crab-shell.

In some sections which I prepared with Ramon y Cajal's silver nitrate method, and which were taken from an unerupted human premolar, a further stage in the consolidation of the matrix is revealed, which appears to throw a strong light on the mode of calcification of dentine and to make clear the meaning of certain appearances in the adult tissue of which I had not hitherto met with any explanation.

As previously stated, the calcospherites seen in human dentine appear clear and structureless, but it was pointed out by Rainey, referring to calcification of the clear calcospherites in shell, that "as the development progresses the globules lose their bright and structureless character and begin to present laminæ and radiating lines

just as the artificial calculi do, the lines *being more distinct when the globules are suffering disintegration*" (3).

The drawings on Plate 7 show that this is the case in the dentine. The decalcified calcospherites are seen to have first coalesced into larger bodies and then to have become laminated, showing strongly marked concentric striæ. The figures in Plate 7 are from the dentine of this tooth, which is stained a deep brown and is everywhere traversed by these contours. In fig. 6 the general appearance of the dentine under a high power is represented. It will be seen that the circular bodies are of very various sizes and that the outermost striæ composing the rings are drawn out into laminæ which pass more or less parallel to the surface of the dentine. This figure shows very clearly this drawing-out of the marginal striæ into lines which remain equidistant from one another. A very perfect concentric body is shown in fig. 7, which illustrates the formation of an interglobular space. Here the spreading of the layers has been arrested and the rounded mass projects into the remains of the uncalcified substance.

Fig. 10 shows that the dentine in a thin section splits along these lines of lamination, the splitting not only following the extended laminæ but also the contours of the round bodies themselves where they have not been drawn out in this manner. It will be noticed that the lines remain absolutely parallel and never join with one another in the same layer; where there is any confusion of the lines it is due to one layer lying over another. The lines in the dentine remain as distinctly separated from one another as in the circular bodies themselves. This form of lamination of the dentine would thus appear to have nothing to do with physiological lines of growth, but to be due to a purely physical cause, the extension of the elements of the globular bodies in parallel lines. In teeth in a further stage of development the rounded contours are to a very great extent lost, and only the parallel striæ remain as evidence of the original structure. This is shown in fig. 8 in a drawing from a section of an adult carious tooth at the border of the carious cavity, where the slight action of the lactic acid produced by the bacteria has revealed the original structure. This appearance I had several times noticed near carious cavities, but had not understood its meaning. In the light of the above observations, however, it is quite evident it is due to the same conditions. It would appear that as the dentine becomes increased in thickness by addition to its growing margin, pressure is exercised on the round bodies and they tend more and more, as the dentine thickens, to lose their globular form and be extended into laminæ.

From these observations it would appear that the stages in the calcification of the dentine are :—First, the appearance of the small clear globular bodies which form in a colloidal matrix by the coalescence of minute particles, as seen in artificial experiments. Secondly, these clear bodies further coalesce, and when thoroughly incorporated in the basis substance of the dentine, become still more completely fused together, lose their structureless character, and exhibit concentric lines. Lastly, the

coalesced calcospherites undergo disintegration, their concentric elements being spread out into the laminæ of the dentine and their lime salts becoming equally diffused in the calcified matrix.

In fig. 5, Plate 7, I have drawn a calcospherite formed in one of the albumen experiments, to compare with those in the dentine on the same plate. This example shows precisely similar concentric markings in the calcified spherite to those in the calcoglobulin substance in the dentine; it also shows very faint indications of radial lines which cannot be detected in the dentine.

Calcification in the Enamel.

Many observers have described globular bodies in the ameloblast cells, ARNELL (6), GRAF SPEE (7), VON EBNER (8), ANDREWS (9) and LEON WILLIAMS (10) having especially drawn attention to these bodies, which are indeed very evident in many specimens of developing enamel. These authors are of opinion that they are the calcifying elements separated by the cells; but others, while quite convinced of the appearance of these bodies, do not feel in a position to offer any positive opinion as to their nature. VON EBNER (8) shows a drawing of the spherical bodies in the newly formed enamel prisms, and considers they are deposited from salts dialysed through the membrane present on the surface of forming enamel. I have, myself, preparations showing these minute globules over a large surface of the young enamel.

LEON WILLIAMS (10) describes an outer and an inner ameloblastic membrane, and appears to consider that a dialysis of lime salts takes place through these membranes. I have given elsewhere in this paper my reasons for considering that these membranes actually exist after calcification has commenced.

In the endeavour to investigate the actual conditions under which the deposit takes place, I took some developing molar teeth from the crypts of a dried skull of a young kangaroo (*Macropus rufus*), in which over the cap of dentine a rough deposit of enamel only, was laid down. Small pieces of this substance were removed with a knife and placed in a drop of glycerine on a slide under the dissecting microscope. The enamel at this stage is very friable, and a gentle touch with the needles separates each small fragment into numerous parallel laminæ. These laminæ are at an acute angle to the surface of the enamel and are perfectly parallel. They can be divided again and again into very thin layers and all lie in the same plane.

With a higher magnifying power it is seen that crossing the rows of prisms at an angle is a delicate fibrillar substance which can in several places be seen projecting on either side of the prisms. VON EBNER shows a similar appearance in a figure in his paper on enamel. This seems to be a delicate fibrillar substance derived from the ameloblasts, and covering the already calcified, or partially calcified, laminæ. These laminæ are arranged like the leaves of a book, apparently interleaved by the delicate membrane described above (Plate 8, fig. 1). Examination with a high power showed that many of these fibrillar interprismatic layers

are covered with calcospherites of various sizes (Plate 8, figs. 2 and 3). Some of these bodies are very large and show radial markings, minute granules, and a radial splitting of the calcospherite into three or four portions. They are surrounded by small globular bodies, which are also seen extended along the laminæ in their neighbourhood and apparently result from the disintegration of the larger bodies. In many places the separated laminæ are seen to be quite covered with these small globules at their edges (Plate 8, figs. 4B and 8).

It was somewhat surprising to find calcospherites of such large dimensions in this situation and to notice their strong resemblance to the artificial bodies produced in albumen (Plate 8, fig. 5). These large spherites appear to be undergoing disintegration and are breaking down into smaller globules.

In a tooth further advanced in calcification from the same animal, but which was not quite erupted, a later stage in the deposition of the calcific material could be studied. In this tooth, in which the enamel was quite smooth and polished on the surface, the consolidation of the substance was still incomplete; very small round globules are seen within the prisms and in the interprismatic substance a long way into the enamel.

It is noticeable that these large calcospherites in enamel show but the very faintest traces of concentric lamination, although they have very distinct radial markings. In dentine, on the contrary, one can see no trace of radial markings, although the concentric rings are very conspicuous. The spherites in enamel, at all events at the stage in which they were here examined, are markedly granular and have not the clear glass-like appearance of the artificial spherites, nor are they so clear as those seen in the early stages of dentine calcification; and this granularity, as described by RAINEY in artificially produced calcospherites, is an indication of disintegration of the globules. The large spaces visible in some of them also show the same condition, and in one case I found a large spherite broken into four segments lying close together.

The meshes of the delicate fibrillar matrix in which these large spherites are deposited are probably filled with the albuminoid matter which LEON WILLIAMS considers to be poured out by the ameloblast cells, and we must suppose that the salts are dialysed into this collagenous organic substance and there form the calcospherites, as in the experiment with albumen devised by Prof. HARTING.

In my slides of the first formed enamel, fibres can be very distinctly seen in many places, crossing the prisms at an angle; and the whole of the enamel, prisms, and prismatic substance would seem to be laid down in this network of fibres.

It is not always the fact that all traces of this substance are lost in calcification, for in some cases this fibrillar basis material can be seen in the completed enamel of marsupials. In two instances in which I succeeded in staining the enamel very completely, in the one case with fuchsin, and in the other with silver nitrate, it can

be seen that small portions of the fibrillar matrix have escaped calcification and have taken the stain deeply (Plate 8, fig. 7).

The fibres are seen crossing the stained tubes in the tubular enamel exactly as in the unstained teased preparations of the developing tooth of the kangaroo. Although it has been often stated that the enamel, once formed, undergoes no further change, I think there can be little doubt, as shown by this observation and many others, that the enamel when formed and functional is not fully consolidated. PICKERILL (11), as the result of his experiments, states that " the degree of penetrability of enamel to stains from the surface bears an inverse ratio to the time the tooth has been erupted," and suggests that an after-hardening of the enamel may take place by the dialysis through Naysmith's membrane of salts from the saliva. As Prof. HARTING (4) says : " La solidification de la substance secrétée est toujours un phénomène secondaire, qui exige un certain espace de temps, pendant lequel la sécrétion continue."

The larger calcospherites which lie in this fibrillar layer are rounded bodies with a somewhat irregular margin ; they show radiating lines and many are finely granular. In many parts the minute spherites of more or less uniform size, which appear to result from the breaking up of these larger bodies, are seen to be extended in lines, diagonal or straight, and often arranged as a layer of minute globules on the surface of the leaf-like expansions. The torn margins of the fragments of enamel are often seen to be crowded with globules of various sizes, and often large masses of coalesced spherites of irregular form are seen projecting from their edges. Simple and compound spherical bodies are also seen lying free in the glycerine in which the fragments of enamel are examined (Plate 8, fig. 4A).

I made several examinations of human developing teeth to see if the same conditions existed as in the teeth of marsupials, but was at first unsuccessful in finding the large calcospherites, although in these teased preparations many prisms showed the lines of tiny globular deposit within them. Having at length obtained some suitable material I found that similar bodies were present to those found in marsupial enamel, and I think there is strong evidence that here also the calcification of the cement substance is due to this deposit. The drawings on Plate 8 are from preparations in glycerine procured in the same manner as those from the marsupial teeth. Most of them are from the developing teeth removed from the tooth sac in the jaw of a 10 months old subject and preserved for a few weeks in spirit. By dividing one of these developing teeth vertically I was able to take minute scrapings with a sharp knife perpendicular to the surface of the enamel at its outer margin, and found that the larger bodies lay for the most part both upon the surface and just beneath the outer clear layer of the enamel within its substance.

The illustration in Plate 8, fig. 9, shows two large bodies in this latter position, and a comparison with the drawings of marsupial enamel will, I think, indicate that these large bodies in human enamel probably bear the same relation to calcification

of the cement substance as do those of the kangaroo. The difference between the two enamels appears to be that in the marsupial, where the organic matrix is more developed and probably less calcified, the spherites can be seen deep in the enamel substance, but in human enamel, where this material is less marked and sooner calcified, the larger calcospherites are only found in the forming layers of enamel near the surface. The large mass of globules of various sizes shown in Plate 8, fig. 12, was found on the surface of the enamel between the developing cusps of a tooth in which the enamel and dentine formed a small open cap over the pulp. These large bodies, which were seen to react to polarised light as do the artificial spherites, would appear to be deposited directly under the inner ameloblastic membrane of the enamel organ, where they have probably formed as the result of the dialysis of salts from the ameloblasts, or the fluid by which they are surrounded. It is evident that these bodies cannot have been present, as such, in the ameloblast cells, many of them having a diameter covering four or five enamel prisms; but they are of all sizes, from this to very minute clear globules. In this situation it is noticeable that, as at the margin of the forming dentine, the spherites are clear and show very little indication of structure; they are highly refractive and by careful focussing are seen to be globular.

Many of the isolated bodies show denser portions in places, some few a faint indication of striæ, and here and there a sphere is found breaking up into four segments (Plate 8, fig. 4c) as in the kangaroo. Many of the small globules have a central prominence like the boss of a shield (Plate 8, fig. 13a). Many of the larger ones are oval, a form I have not met with in the marsupials, and a few are strongly granular; these last appear to be disintegrating and do not react to polarised light.

In looking for these bodies in developing enamel it is very important to make use of polarised light, as by this means they can with certainty be distinguished from other substances, such as leucocytes, which have found their way in from the tooth sac. I think it is very probable that the stage of calcification in which these large calcospherites are found is a very passing one; the larger bodies very quickly breaking up, for in the enamel of the kangaroo I have found that in slides prepared from the same part of the tooth, three or four of the laminæ will be crowded with the round bodies, and 20 or 30 other fragments in the same preparation may show none.

The actual presence of large calcospherites in developing enamel other than the regular globules in the prisms has, I believe, not been hitherto described. Prof. Underwood, however, described interglobular spaces in human enamel, in 1896 (12), in some cases of erosion of the enamel. The globules surrounding the spaces are very small, but have much the same appearance as in the interglobular spaces in dentine. It would appear that in these pathological teeth the action of the agents (whether acids or not is disputed) had just reached a stage in which the process

of development of the enamel was revealed, in the same manner as the acids of caries reveal the structure of the calcospherites in dentine. They were only seen in these cases of erosion. We can hardly, however, look upon these as corresponding to interglobular spaces in dentine, which are due to an interruption of calcification during the formation of the tooth, and if there were a similar interruption in the enamel deposit we should expect to find these spaces in healthy teeth, as is the case in dentine.

One would feel more disposed to consider that the erosion process had partially dissolved away some of the lime salts of the matrix in an irregular manner, leaving the presumably denser portions represented by the globular deposit.

The first large calcospherites which I have described were found in marsupial enamel, which from its somewhat primitive condition affords a good opportunity of studying the early stages of the process, which are very much obscured in the more calcified enamel of human teeth, although I have shown that a similar deposit takes place here also.

The prisms, or, at all events, the greater part of their substance, appear to be the first calcified material formed, probably by the deposit of lime salts in the Tomes' processes of the ameloblasts.

The lime salts are deposited in the interprismatic substance as calcospherites, probably by the formation of nascent carbonate and phosphate of lime within this substance by the chemical combination with its contents of salts dialysed through the inner ameloblastic membrane, or the outer surface of the colloidal matrix, which such membrane would represent.

In marsupial enamel, as I have shown above, calcification takes place in the laminæ between the prisms, and I think it is very evident that these large calcospherites are produced quite independently of the prisms upon which they lie. The prisms are calcified in rows of minute spherical bodies, which appear to be formed within the Tomes' processes of the ameloblasts, and are the first part of the enamel to receive the calcific deposit, while the interprismatic substance is formed subsequently in the organic fibrillar matrix, which is seen throughout the enamel in the kangaroo. The calcification in marsupial enamel does not, however, proceed to complete obliteration of the cement substance, much of which remains uncalcified, as the alcoholic fuchsin method clearly shows.

It would appear that in less perfectly formed enamels a cement substance is very evident, and that the denser and more completely formed the enamel, the less is this substance in evidence, its existence being veiled by calcification, so that in the enamel of the Primates the evidence of its presence in the perfectly formed portions of the tissue is very slight or altogether absent.

In explaining the calcification of enamel and of dentine by the deposit of lime salts in the form of calcospherites we are met with one great difficulty. The analyses of dentine and enamel show a very large preponderance of phosphates over carbonates.

In dentine, according to the analysis of BERZELIUS, phosphates form 62 per cent. of the mineral matter and carbonates 5·50 per cent. In enamel VON BIBRA'S analysis gives for adult enamel 89·82 calcium phosphate and fluoride, and only 4·37 of calcium carbonate.

As pointed out in a previous part of this paper, whenever in artificial experiments the phosphates are in excess of the carbonates, the lime salts are deposited in a crystalline form, and not as globules, although the presence of a small proportion of phosphate combined with a large amount of carbonate gives the best and most perfect calcospherites. Mr. F. J. BENNETT (13), in a paper on calcification in the teeth, says: "Nowhere, as far as I know, does direct experiment favour the globular form as a possible arrangement of the lime salts in the teeth." No doubt it is very difficult, perhaps impossible, to reproduce by experiment the exact conditions in living organisms, but this does not necessitate the abandonment of the view that lime salts are deposited in the dental tissues in this form. In the present paper I have not supposed that any experiment out of the body can exactly reproduce the natural process, but I think the general principles on which the physical process of calcific deposit takes place are well shown by the experiments of RAINEY and HARTING, and by the formation of calcified structures in the mollusca and crustacea.

I have, however, shown that calcospherites which exactly resemble many of the forms artificially produced, *are* found both in dentine and enamel, and we have to meet this fact, and consider how it is that calcification does occur in this form, despite the chemical composition of the finished tissues.

I think we must look for an explanation in the composition of the enamel and dentine in early stages of development, although unfortunately the difficulties in the way of obtaining any reliable analysis of the first formed enamel and dentine appear to be insuperable.

It may, however, well be, as pointed out by Prof. SIMS WOODHEAD (14) that, as in the early stages of bone formation, the carbonates may be present in excess. In speaking of bone, he says: "Newly formed bones, or new bone-tissue of any kind, where the cells are extremely active in building up the matrix, almost invariably have a larger proportion of carbonate of lime than fully formed bones, because here the active cells set free a larger proportion of carbonic acid, as a result of which more phosphoric acid may be replaced by the carbonic acid lime salts." The same conditions probably apply to the other calcified organic tissues of the body, as enamel and dentine. The conditions in young growing animals, where the metabolic changes are very active, are eminently favourable to the production of carbonic acid, and it is quite conceivable, and in fact probable, that in the early stages of enamel and dentine formation, the carbonate may be greatly in excess of the phosphates, and thus the deposit of lime salts as calcospherites be easily understood. The after alteration of the chemical composition of the material in which calcification occurs, and the addition of salts of phosphorus from the blood, would perhaps serve to account for the

breaking up of the calcospherites as calcification advances and the ultimate hard condition of the dentine, and the dense semi-crystalline aspect of the enamel in its fully completed state.

It is evident from numerous experiments, especially those of HARTING and ORD, that in the proportions found in finished bone and teeth the lime salts are not deposited in albumen in the globular form, but we do not know the exact chemical constitution of the exuded material in which this deposit takes place in the living body, and as stated by Mr. TOMES (15, p. 55) HOPPE-SEYLER considers that the salt in teeth and bone is a double salt, consisting of three equivalents of calcium phosphate with one of calcium carbonate. Whether in this combination the influence of the carbonate may be effective in causing a globular deposit might be considered, but in whatever way we explain the chemical conditions which allow of their formation, we cannot escape the evidence that in both dentine and enamel the lime salts are deposited in the globular form.

With regard to the other mineral constituents of enamel, the fluoride of calcium is present in very small quantity, but magnesium salts are present in the proportion of from 1·34 to 2·55 per cent. Mr. BENNETT mentions an experiment of RAINEY'S in which magnesium phosphate was added to gum solutions of carbonate in similar proportions and in which no globular bodies were produced, but Prof. HARTING describes an experiment in albumen to which a magnesium salt was added, and in which he found that the precipitate was composed of the same bodies, as to form and structure, as those which had formed without the addition of magnesia; the only effect he remarked was that the formation of the precipitate was delayed.

We thus see that in both dentine and enamel the lime salts are deposited in the spherical form throughout, the larger forms eventually breaking up and being lost in the calcified mass. We have, as in RAINEY'S experiments, a coalescence of the small globular particles leading to the production of the large calcospherite, followed by disintegration of these forms and a deposition in the formed tissue in a more homogeneous condition. As pointed out by RAINEY, certain conditions accelerate the process of disintegration. He found that when calcospherites consisting of carbonate and phosphate were placed in a denser medium than that in which they were produced, they became rapidly disintegrated, but he did not find this occur with those formed of carbonate of lime alone. A fresh deposit of spherites upon those already formed, also caused disintegration of those first deposited.

In some experiments which I undertook for the purpose, I found that the addition of crystals of phosphate to albumen containing already formed calcospherites of carbonate, prevented their further formation and caused disintegration of those already formed.

It is, therefore noticeable that the salts of lime in the condition of calcospherites are not in a very stable state of equilibrium, they alternately coalesce and disintegrate under the effect of various conditions in the medium in which they are produced.

This fact has an important bearing upon their varying conditions of aggregation in the calcifying structures of living organisms.

The Sheath of Neumann.

The existence or non-existence of this sheath has long been a matter of controversy. JOHN TOMES* was the first to describe the presence of a soft fibril in the dentinal tubes. He showed that these fibrils ran in tubules in the dentinal matrix, and were provided with a definite lining membrane; the sheath first fully investigated by NEUMANN (16) and named after him. This lining membrane shows a great resistance to the action of acids and can be isolated in preparations of dentine by the destruction of the intervening matrix substance by the prolonged operation of strong acids.

Some observers, however, have considered that the appearance of a lining membrane to the dentinal canals is due to effects of refraction, and MAGITÔT (17), SUDDUTH (18), and, more recently, RÖMER (19), deny its existence. Mr. CHARLES TOMES says: "It must be remembered that the dentinal sheaths can only be fully demonstrated by processes which amount to a partial destruction of the dentine, and that they are therefore in some degree, at all events, artificial; it may be they have no real existence until they are called into existence by the action of these agents." He has, however, succeeded in staining them with nigrosin, and RÖSE with silver nitrate, and I have succeeded in doing the same in many preparations.

Prof. RÖMER considers the tubes to be simply spaces in the dentinal matrix with no true walls; the so-called Neumann's sheath he affirms to be a part of the soft fibril, and considers that in the preparations in which it is claimed that the sheath is stained, what is really stained is the outer surface of the soft fibril. He shows a figure in the illustrations to his paper on the subject, in which in transverse section the fibril has fallen out, and there is a perforation in the dentine with no wall to it. Judging by the illustration it would seem probable that in this instance the fibril only is stained, and the sheath not, as I have several preparations which show an outer ring enclosing another dark stained ring, which is the border of the dentinal fibril.

It is somewhat difficult to obtain good staining of the Neumann sheath, my most successful preparations having been from young teeth stained by a modification of the method of RAMON Y CAJAL. In these preparations the sheath of Neumann is stained a deep black, and the dark-stained fibril is seen lying within the ring, an appearance which cannot be explained on the assumption that the supposed sheath is the outer border of the stained fibril. Plate 7, fig. 1, shows this very distinctly, and, moreover, at (*aa*) gives still more definite proof of the existence of the sheath, for at the thin margin of the section the segments of circles are seen

* 'Phil. Trans.,' 1853.

surrounded by a black-stained line, and the stained fibril has fallen away altogether. It does not seem possible to account for this appearance at the margin of the section in any other way than as a definite proof of the actual existence of the structure called the sheath of Neumann, and, moreover, of its existence as an integral part of the dentine lining the tube.

The tube section shows a sharply defined line, there being no diffused staining of the surrounding matrix, showing that this wall consists of some material which takes the stain quite distinctly from the rest of the matrix. In the specimen in which the segments of the tubes are seen and the fibril has entirely fallen away, there is no possibility of any disturbance of the image from the thickness of the section or from optical refraction. The sheath is also very evident in the projecting ends of tubes which are cut obliquely (Plate 7, fig. 2).

In Plate 7, fig. 3, it is seen, as was pointed out by ERWIN HÖHL (20), that in longitudinal section, while the sheath of Neumann is very evident, with suitable staining, in the calcified portion of the dentine, it is not to be seen in the odontogenetic zone where the tubes appear to have no definite walls. The figure shows this very distinctly in a section of a young growing tooth where the uncalcified zone is of great width, the section being a portion of the same preparation as that from which fig. 1 was taken. HÖHL considers that this fact points to "the dependence of the sheath of Neumann on calcified dentine substance." We might perhaps carry this statement further and suggest that this sheath of the tubes is concerned in the calcifying process, and may serve as a dialysing membrane through which dialysis takes place.

In developing enamel a membrane has been described between the ameloblast cells and the forming enamel, and also between the enamel cells and the stratum intermedium of the enamel organ. The existence of membranes in these situations has been the subject of much controversy.

Mr. CHARLES TOMES is inclined to look upon these membranes as artificial productions, but in sections which have not been treated with either alcohol or acid they are easily to be seen. At the base of the tooth-germ, where the formation of enamel has not yet commenced, the inner membrane cannot be seen, and the outer ameloblastic membrane is evidently quite absent, as the cells of the stratum intermedium are mingled with the ameloblasts (Plate 8, fig. 6), although after the commencement of calcification they are distinctly separated as a layer of cells, lying for the most part more or less at right angles to the ameloblasts. They certainly appear to be separated by what seems like a continuous membrane.

Further evidence of the existence of membranes in these situations is given in Plate 8, figs. 10 and 11.

Fig. 10 shows the outer ameloblastic membrane of the enamel organ of *Macropus* from which the ameloblasts had fallen away, and the cells of the stratum intermedium are seen to be separated by a definite membrane from the region of the

ameloblasts. In two other specimens I found a similar separation of the cells from the inner ameloblastic membrane.

Fig. 11 represents a small portion of the dried up enamel organ of *Macropus* which was found with the fragments of enamel in the teased preparations in glycerine previously described. This appears to represent the inner ameloblastic membrane and the membrane of the honeycomb connected by the shrivelled Tomes' processes, and would appear to be strong evidence of the existence of these structures.

With regard to the sheath of Neumann in the dentine the fine branches of the dentinal tubes everywhere traverse the dentine and must either penetrate the sheath or be themselves provided with a similar sheath. It is very difficult to determine this point in such minute structures, although their sharp outlines might suggest that the latter is the case.

With respect to the dentine the arguments which would appear to support the view stated above are : the evidence of the real existence of the sheath of Neumann, its absence in the layer of collagenous material in which calcification is about to take place but has not yet commenced, and its very conspicuous presence in young growing dentine. It also suggests a function for a structure, the uses of which have not been understood.

In growing dentine, as is well known, the calcified dentine is separated from the odontoblast cells by the odontogenetic zone, consisting of a substance considered to be elaborated by the cells of the pulp, and upon which the calcific matter encroaches from above in rounded contours.

This calcific deposit never proceeds direct from the odontoblasts, and it has been a little difficult to understand why calcification takes place only in this direction, but on the view that the lime salts conveyed by the fibril are dialysed through the Naysmith's membrane into this collagenous material it can, I think, be more easily understood.

Conclusions.

I have endeavoured to show that calcification in both dentine and enamel is in great part a physical phenomenon ; that the actual deposit in both tissues occurs in the form of calcospherites, and that the process in mammalian tissues is identical in many points with the same processes occurring in lower organisms.

While no doubt it is true that no artificially conducted experiment can accurately represent what occurs in the living body, the resemblances between the substances produced in these experiments and those found in the dentine and enamel are very strong, and the objections to this comparison have been, I think, chiefly based on the chemical difficulty, the composition of the dentine and enamel not being such as to allow the assumption of the spherical form in the deposited lime salts. As I have, however, endeavoured to show, the spherical form is not retained in completed well-formed dentine or enamel, and for the reasons given it is highly probable that when the lime salts are deposited in the colloid material of the matrix substance they are

true calcospherites, corresponding to those produced in the experiments, the carbonate of calcium being combined with a small proportion only of phosphate.

That some altered chemical conditions of this nature must occur is, I think, evidenced by the fact, which I have described above, that large calcospherites comparable in every respect to those found in the organic solutions employed in the experiments are to be seen in young enamel. These large bodies only persist in this state of aggregation for a short time, and as phosphates are added from the blood they break down, and the resulting disintegrated particles form the finally consolidated enamel.

In dentine also, except where the process of calcification has been arrested at the interglobular spaces, a similar breaking down of the first formed calcospherites takes place, the only evidence of their former presence being the lamination of the dentine, which is also finally obscured by full calcification in the completed tissue.

The evidences that the odontoblast cells are the principal active agents in calcification of the dentine are, I venture to think, quite as conclusive as the similar evidences of the function of the ameloblasts—a function which has not yet been doubted in their case.

As nerve-fibres traverse the dentinal canals, the fibril cannot be looked upon as a transmitter of nervous impulses—the cells have granular contents, they lie in a rich plexus of blood-vessels, and we know that active secretion is associated with an increased blood supply ; like other secreting cells, they are large and well differentiated from the surrounding tissue elements, and they retain their full size and characteristics during the actual deposition of the dentine in healthy teeth.

The protoplasmic prolongation of the cell in the form of the dentinal fibril would be considered to share in the functions of the cell of which it forms a part, and there are strong evidences that calcific matter is transmitted by the fibril. The translucent zone in caries, which a great weight of evidence suggests is due to calcification in the tubes, and the peripheral occlusion of the tubes on exposed surfaces, point to this extension of the cell protoplasm being the channel by which lime salts are conveyed to the dentine.

If I am right in supposing that the sheath of Neumann serves as a dialysing membrane, the comprehension of the process of calcification in the dentine is somewhat simplified. The odontoblast cell, either alone or in common with other cells in the pulp which send processes to the dentine, would deposit the gelatinous basis substance in which calcification takes place—the substance which forms the odontogenetic zone—the lime salts taken up from the circulating blood by the secreting cells would be transmitted by the fibril, and passing by dialysis into the matrix, lay down the calcifying material in the globular form, slow diffusion of the component salts, such as takes place through dialysing membranes, being an important factor in the production of the calcospherites. The presence of large globular calcospherites in the enamel also suggests the slow diffusion of lime salts,

which would appear to be dialysed through the inner ameloblastic membrane, or what is equivalent functionally to such a membrane—the outer surface of the albuminoid material in which the deposit takes place—and, as the matrix substance becomes richer in phosphates, the breaking up of these bodies into smaller elements.

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DESCRIPTION OF PLATES.

Plate 7.

Fig. 1.—Transverse section of human premolar stained with silver nitrate (pyridin process). The sheath of Neumann is stained black, the fibril is also darkly stained. At (*a.a.*) are seen portions of tubes at a thin margin which have lost their contents, but the sharply defined sheath is seen remaining and lining the sections of the tubes. × 1000.

Fig. 2.—From the same preparation. The tubes are cut obliquely, they have lost their contents and show the lining sheath, stained black. × 1000.

Fig. 3.—From the same preparation, at the pulp margin. (*a*) Calcified dentine, (*b*) the odontogenetic zone; the sheaths are seen to be deeply stained at (*a*) and altogether absent at (*b*). × 600.

Fig. 4.—Calcospherites in a decalcified section of a human premolar with uncompleted root. The section was taken at the growing end of the root. × 225. Stain—pyridin silver nitrate.

Fig. 5.—A radial calcospherite—artificially formed in albumen—for comparison with those in the dentine. × 500.

Fig. 6.—A portion of the dentine from a decalcified unerupted human premolar, showing the contours of the calcoglobulin basis of the calcospherites and the extension of their concentric elements into laminæ extending across the dentine at right angles to the tubes (*a.a.*). Stain—Ramon y Cajal's silver nitrate. × 1000.

Fig. 7.—The formation of an interglobular space. A very perfect radial calcospherite projects into the space. Stain as No. 6. × 400.

Fig. 8.—From an adult carious tooth, taken near the margin of the carious cavity, showing the laminæ, which have exactly the same appearance as those in the calcifying tooth. The section was not artificially treated with acids, what decalcification has taken place being due to the acids of caries.

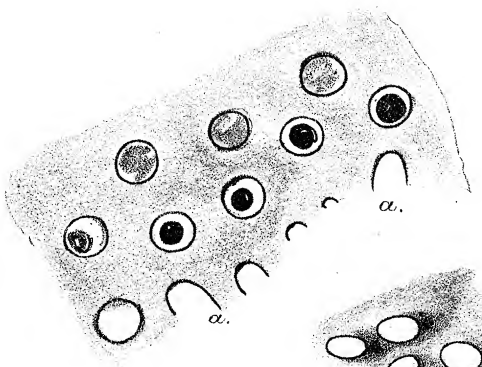
Fig. 9.—From a similar preparation to No. 6, showing a central apparently denser body enclosed in the concentric lines of another calcospherite. × 800.

Fig. 10.—From near the margin of the same preparation, showing the splitting of the dentine along the lines formed by the contours of the calcospherites and their lateral extensions. × 600.

Fig. 11.—Calcospherites in the prawn (*Palæmon serratus*), showing coalescence and disintegration. × 140.

Fig. 12.—Ground section of shell of crab under dark-ground illumination. The striæ in the coalescing calcospherites are seen to be identical with those in the formed shell. (*a*) shell; (*b*) calcospherites; (*c*) fibrillar basis. × 500.

Fig. 13.—Margin of the calcifying claw of *Nephrops Norvegicus*. (*a*) the large spherites disintegrating; (*b*) the fibrillar basis substance. × 140.



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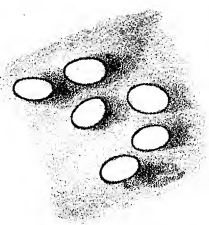

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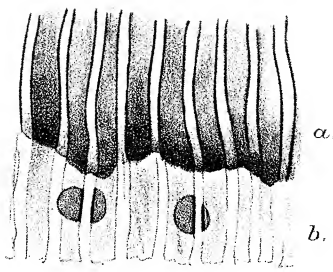

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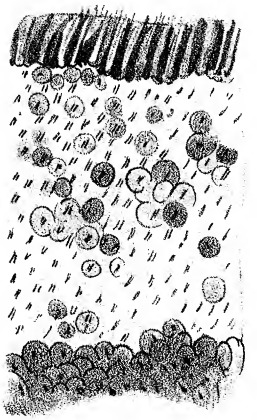

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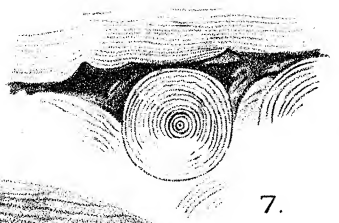

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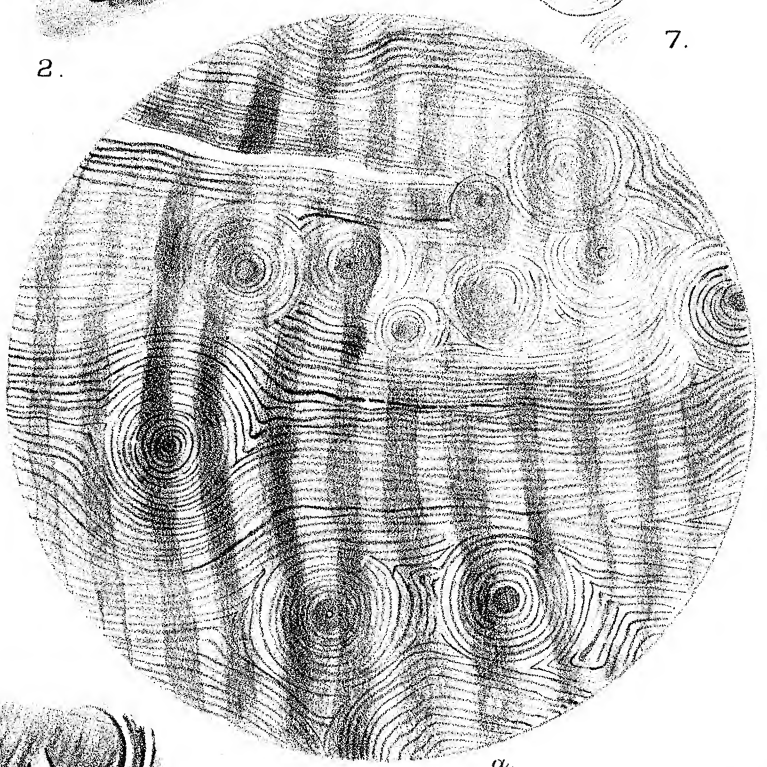

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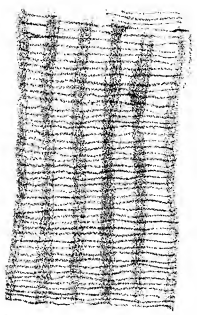

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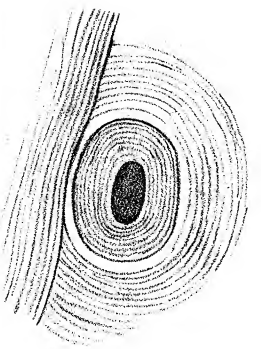

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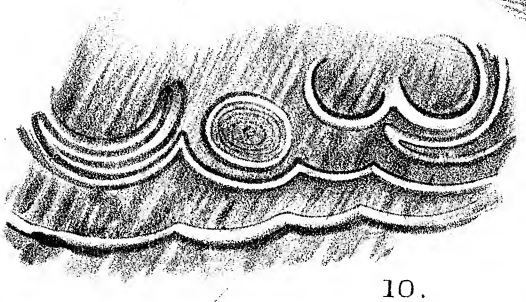

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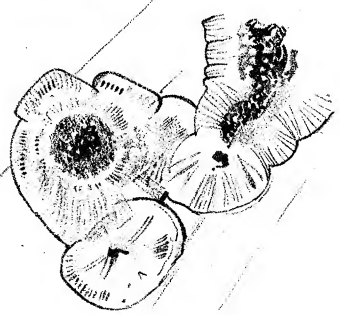

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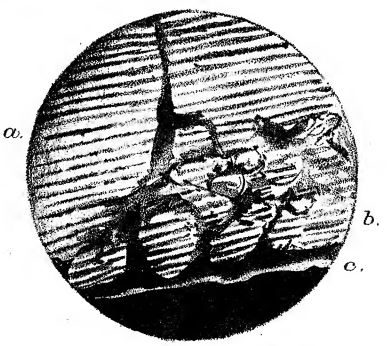

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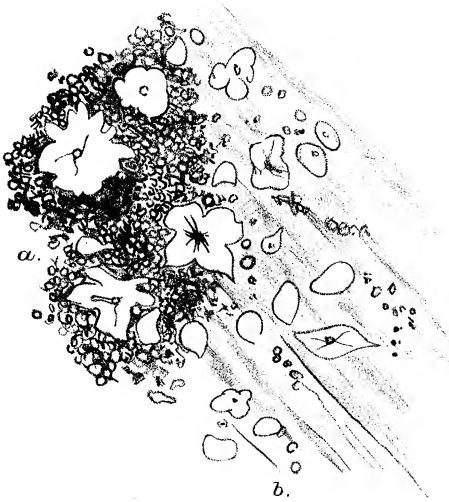

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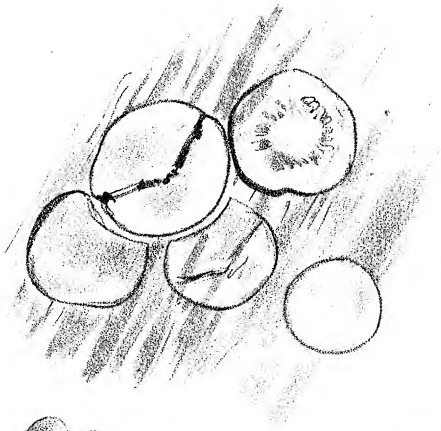

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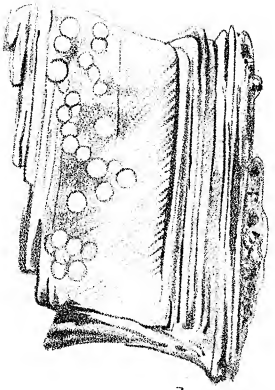

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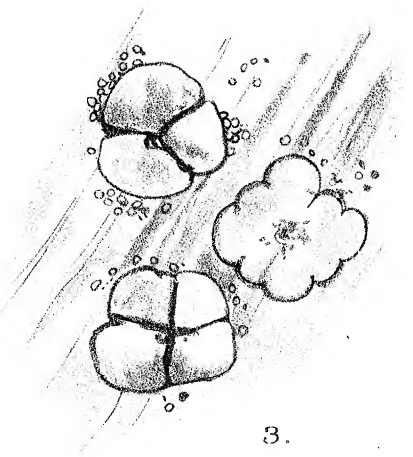

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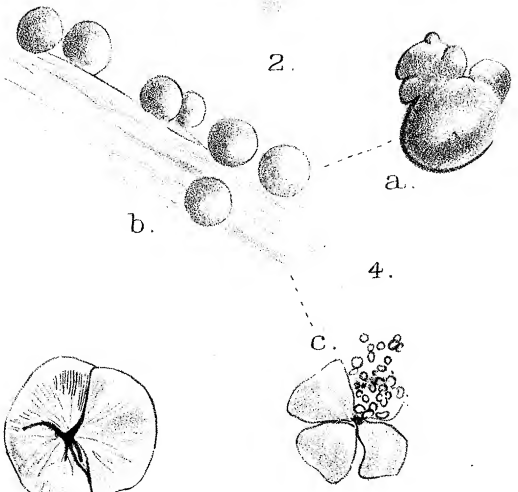

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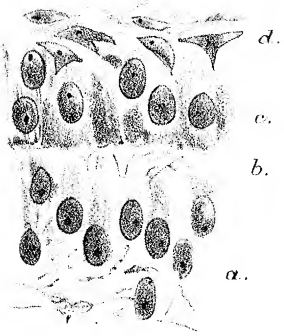

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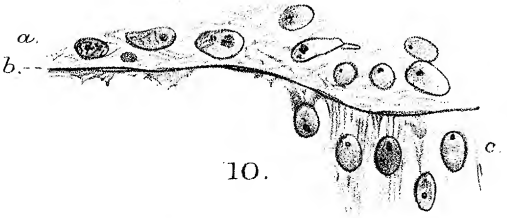

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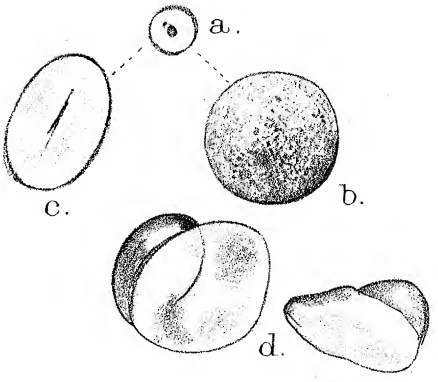

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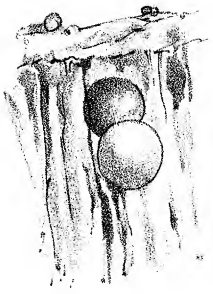

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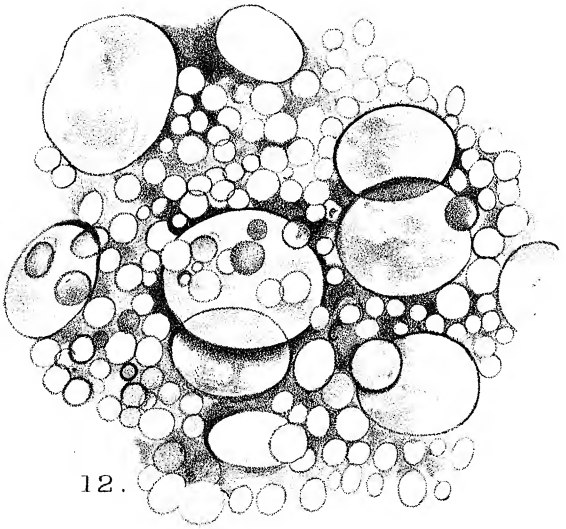

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PLATE 8.

Fig. 1.—A fragment of enamel of *Macropus rufus*, taken from the first formed enamel in the crypt in a dry preparation. The fragments teased out with needles in glycerine, showing calcospherites lying in the laminæ into which the enamel separates. × 140.

Figs. 2 and 3.—Large radial calcospherites in the enamel of *Macropus* from the situation in which they are seen in fig. 1. × 1000.

Fig. 4.—(*a*) A large compound spherite found free in the glycerine, (*b*) smaller round bodies lying upon the prisms, (*c*) a radial spherite from human enamel breaking up. × 1000.

Fig. 5.—A radial spherite formed artificially in albumen for comparison with those in the enamel. × 300.

Fig. 6.—Developing tooth of kitten before the commencement of calcification, showing that the cells of the stratum intermedium are mingled with the ameloblasts, no membrane being visible. (*a*) cells of the pulp, (*b*) fine fibres which become incorporated with the dentine matrix, (*c*) ameloblasts, (*d*) stratum intermedium. × 800.

Fig. 7.—A tube in the enamel of *Macropus*, injected with alcoholic fuchsin from the pulp cavity (method of VON BEUST). The tube is deeply stained, as is also a portion of the fibrillar matrix which has escaped calcification. × 800.

Fig. 8.—A row of small spherites in the interprismatic substance. × 500.

Fig. 9.—Globular bodies in human enamel, in the layers of enamel just beneath the clear outer portion. Shown by polarised light to be calcified. × 500.

Fig. 10.—The outer ameloblastic membrane of the enamel organ of *Macropus* from the under side of which the enamel cells have fallen away. (*a*) Stratum intermedium, (*b*) membrane, (*c*) ameloblasts. × 800.

Fig. 11.—A portion of the dry enamel organ of a teased preparation of enamel of *Macropus*. (*a*) line of inner ameloblastic membrane; (*b*) membrane of honeycomb. × 300.

Fig. 12.—A portion of a mass of calcospherites, reacting to polarised light lying upon the forming enamel after removal of the enamel organ, human developing tooth. × 700.

Fig. 13.—Forms of calcospherites found in human enamel. (*d*) A form frequently seen probably due to the union of two oval spherites (*c*), (*b*) a calcospherite which is finely granular. × 700.

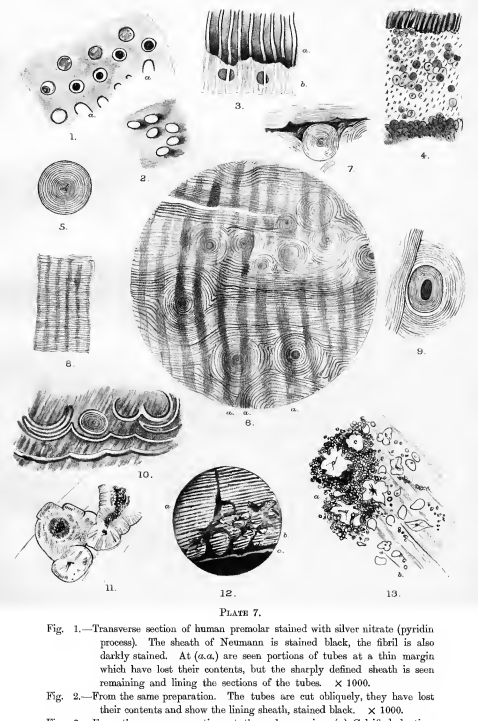

PLATE 7.

Fig. 1.—Transverse section of human premolar stained with silver nitrate (pyridin process). The sheath of Neumann is stained black, the fibril is also darkly stained. At (*a.a.*) are seen portions of tubes at a thin margin which have lost their contents, but the sharply defined sheath is seen remaining and lining the sections of the tubes. × 1000.

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Fig. 9.—From a similar preparation to No. 6, showing a central apparently denser body enclosed in the concentric lines of another calcospherite. × 800.

Fig. 10.—From near the margin of the same preparation, showing the splitting of the dentine along the lines formed by the contours of the calcospherites and their lateral extensions. × 600.

Fig. 11.—Calcospherites in the prawn (*Palæmon serratus*), showing coalescence and disintegration. × 140.

Fig. 12.—Ground section of shell of crab under dark-ground illumination. The striæ in the coalescing calcospherites are seen to be identical with those in the formed shell. (*a*) shell; (*b*) calcospherites; (*c*) fibrillar basis. × 500.

Fig. 13.—Margin of the calcifying claw of *Nephrops Norvegicus*. (*a*) the large spherites disintegrating; (*b*) the fibrillar basis substance. × 140.

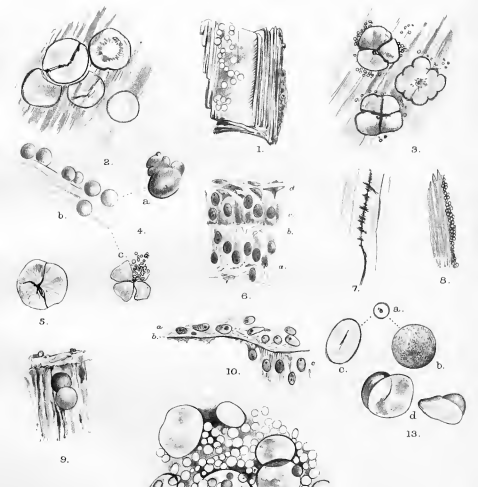

PLATE 8.

Fig. 1.—A fragment of enamel of *Macropus rufus*, taken from the first formed enamel in the crypt in a dry preparation. The fragments teased out with needles in glycerine, showing calcospherites lying in the lamine into which the enamel separates. × 140.

Figs. 2 and 3.—Large radial calcospherites in the enamel of *Macropus* from the situation in which they are seen in fig. 1. × 1000.

Fig. 4.—(*a*) A large compound spherite found free in the glycerine, (*b*) smaller round bodies lying upon the prisms, (*c*) a radial spherite from human enamel breaking up. × 1000.

Fig. 5.—A radial spherite formed artificially in albumen for comparison with those in the enamel. × 300.

Fig. 6.—Developing tooth of kitten before the commencement of calcification, showing that the cells of the stratum intermedium are mingled with the ameloblasts, no membrane being visible. (*a*) cells of the pulp, (*b*) fine fibres which become incorporated with the dentine matrix, (*c*) ameloblasts, (*d*) stratum intermedium. × 800.

Fig. 7.—A tube in the enamel of *Macropus*, injected with alcoholic fuchsin from the pulp cavity (method of VON EBNER). The tube is deeply stained, as is also a portion of the fibrillar matrix which has escaped calcification. × 800.

Fig. 8.—A row of small spherites in the interprismatic substance. × 500.

Fig. 9.—Globular bodies in human enamel, in the layers of enamel just beneath the clear outer portion. Shown by polarised light to be calcified. × 500.

Fig. 10.—The outer ameloblastic membrane of the enamel organ of *Macropus* from the under side of which the enamel cells have fallen away. (*a*) Stratum intermedium, (*b*) membrane, (*c*) ameloblasts. × 800.

Fig. 11.—A portion of the dry enamel organ of a teased preparation of enamel of *Macropus*. (*a*) line of inner ameloblastic membrane; (*b*) membrane of honeycomb. × 300.

Fig. 12.—A portion of a mass of calcospherites, reacting to polarised light lying upon the forming enamel after removal of the enamel organ, human developing tooth. × 700.

Fig. 13.—Forms of calcospherites found in human enamel. (*d*) A form frequently seen probably due to the union of two oval spherites (*c*), (*b*) a calcospherite which is finely granular. × 700.